

Fig. 1 A

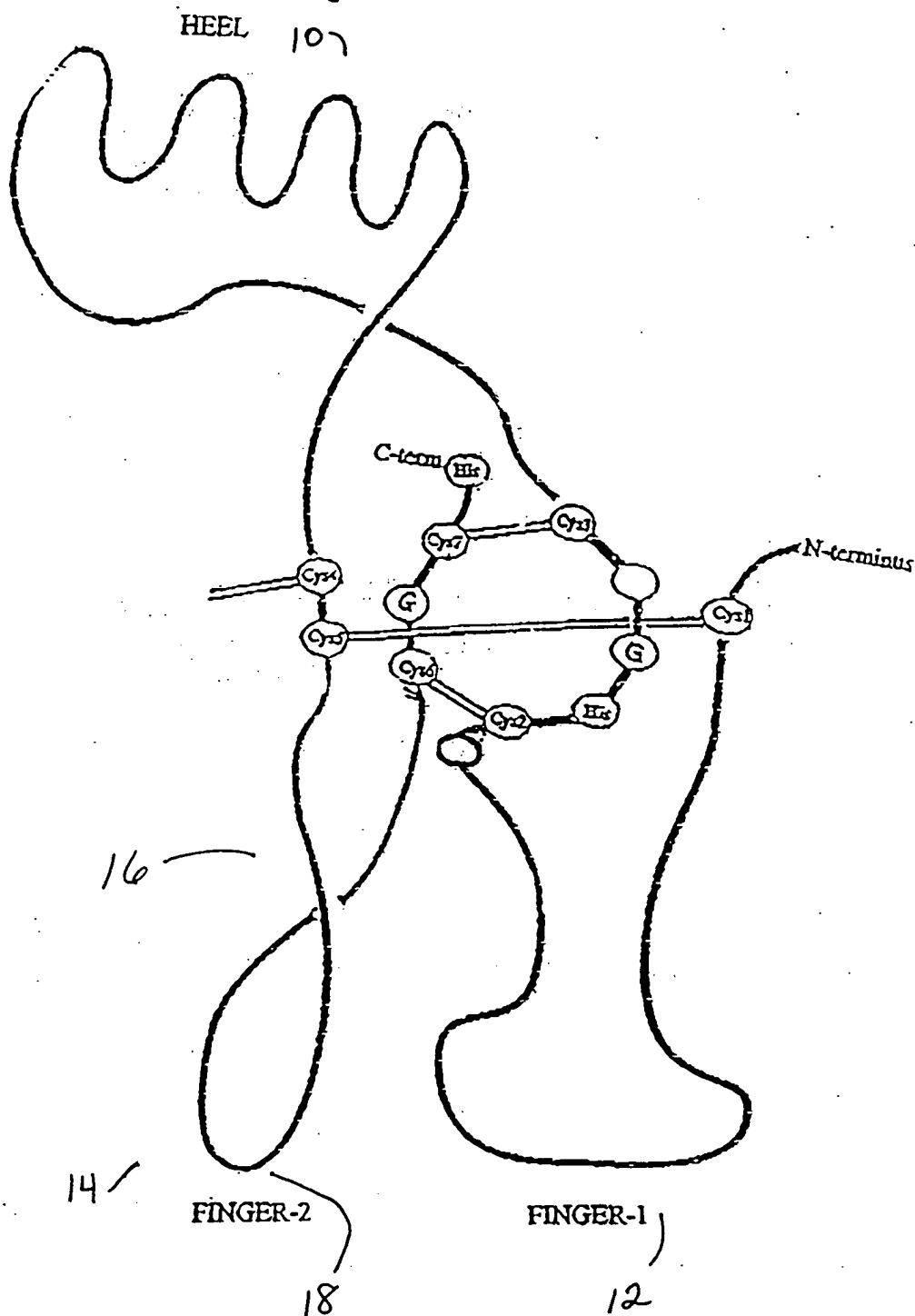


FIG. 1B

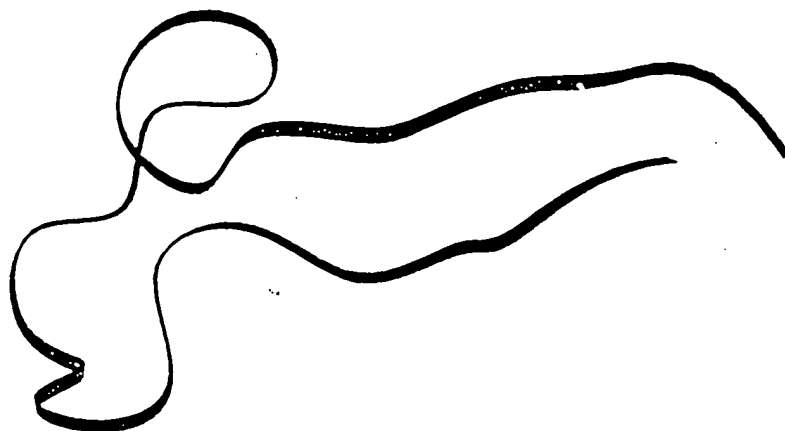


FIG. 1C

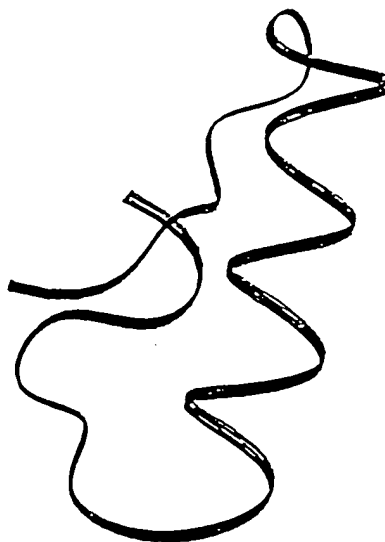
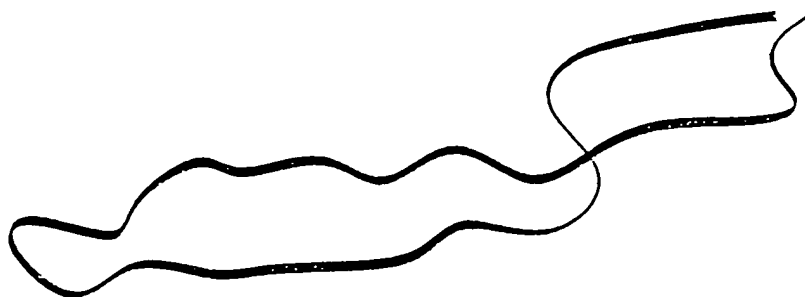


FIG. 1D



09375333-001699

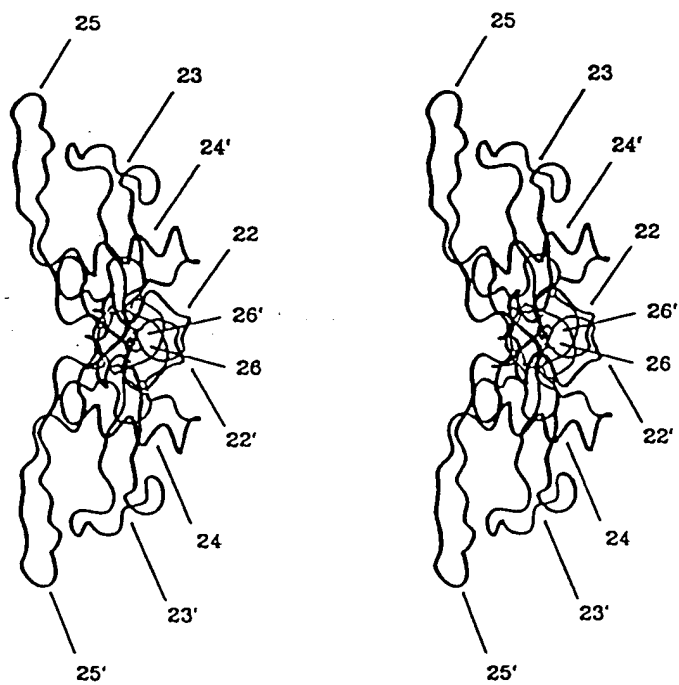
[illegible]

FIG. 3

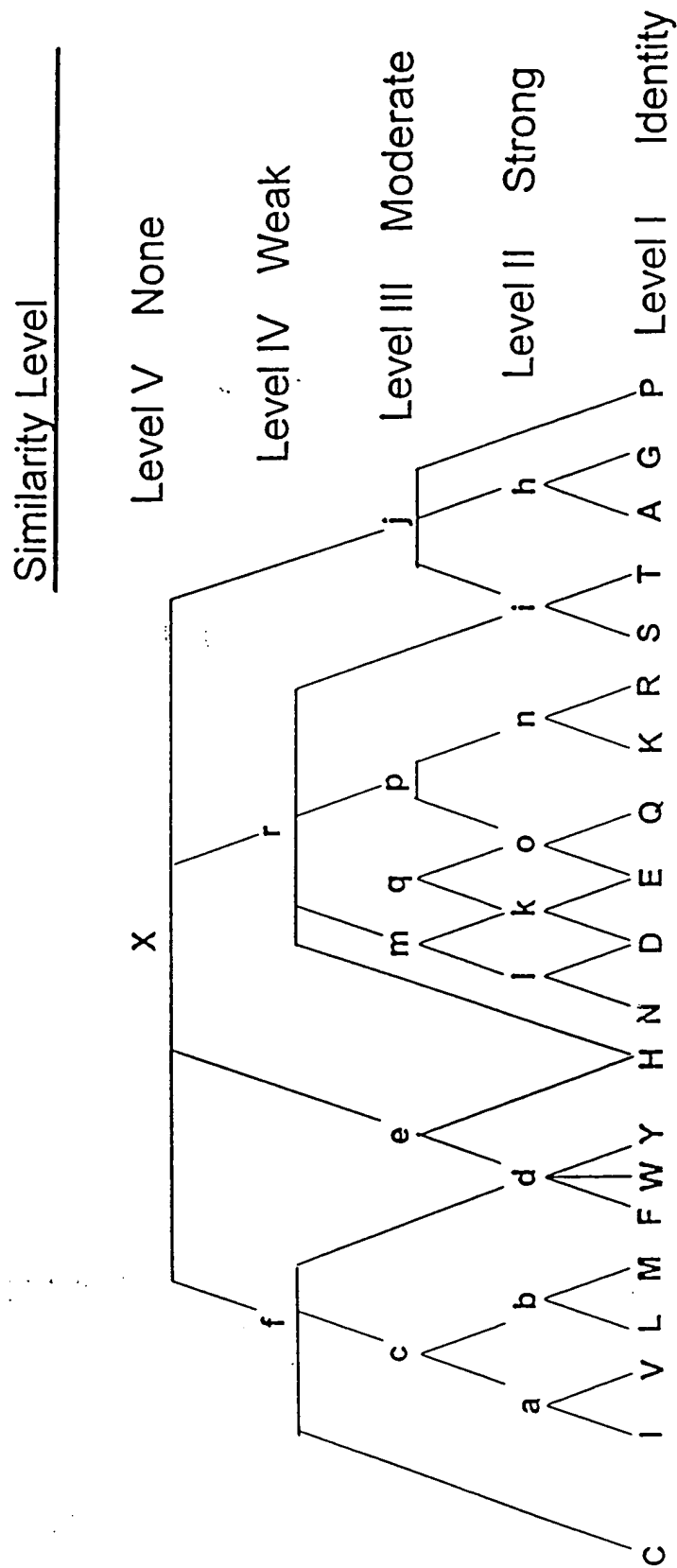


Fig. 4

OP-1	CGA	-	PTQLNAISVLYFOOS	-	SNVILKKYRNMVVRA	CGGH
BMP-5	CGA	-	PTKLNAISVLYFOOS	-	SNVILKKYRNMVVRS	CGGH
BMP-6	CGA	-	PTKLNAISVLYFOON	-	SNVILKKYRNMVVRA	CGGH
OP-2	CGA	-	PTKLSATSVLYYOSS	-	NNVILRKHRNMVVKAC	CGGH
OP-3	CGV	-	PTELSAISLLYYORN	-	NNVILRRERNMVVQA	CGGH
60A	CGA	-	PTRLGALPVLYHLND	-	ENVNLKKYRNMIVKS	CGGH
Vg-1	CGV	-	PTKMSPISMLFYDNN	-	DNVVL RHYENMAVDE	CGGR
UNTMH	CGA	-	PTKLSGISMLYFDNN	-	ENVVL RQYEDMVVEA	CGGR
BMP-2	CGV	-	PTELSAISMLYLOEN	-	EKVVLKNYQDMVVEG	CGGR
BMP-4	CGV	-	PTELSAISMLYLOEY	-	OKVVLKNYQEMVVEG	CGGR
GDF-5	CGV	-	PTRLSPISILFIDSA	-	NNVVYKQYEDMVVES	CGGR
GDF-6	CGV	-	PTKLTPI SILYIOAG	-	NNVVYKQYEDMVVES	CGGR
GDF-7	CGV	-	PARLSPISILYIOAA	-	NNVVYKQYEDMVVEA	CGGR
COMP-2	CGV	-	PTKLTPI SILYIOAG	-	NNVVYNEYEEMVVES	CGGR
6pp	CGV	-	PTOLDSVAMLYLNDQ	-	STVVLKNYQEMTVVG	CGGR
BMP-9	CGV	-	PTKLSPISVLYKDDMG	-	VPTLKYHYEGMSVAE	CGGR
DORSALIN	CGV	-	PTKLDAISILYKDDAG	-	VPTLIYNYEGMKVAE	CGGR
BMP-10	CGV	-	PTKLEPISILYLKDG	-	VVTYKFKYEGMAVSE	CGGR
GDF-3	VG	-	PTKLSPISMLYQOSD	-	KNVIL RHYEDMVVDE	CGGR
GDF-1	CGV	-	PERLSPISVLFFDNE	-	DNVVL RHYEDMVVDE	CGGR
SCREW	CGV	-	PTVLGAITILRYLNE	-	DIIDLT KYQKAVAKE	CGGH
BMP-3	CGV	-	PEKMSSLSILFFDEN	-	KNVVLKVYPNMTVES	CGGR
NOOAL	CGA	-	PVKTKPLSMLYVDN	-	GRVLLBHHKDMIVEE	CGGL
TGF-42	CGV	-	SQDLEPLTILYYIG	-	KTPKIEQLSNMIVKS	CKGS
TGF-43	CGV	-	PQDLEPLTILYYVG	-	RTPKVEQLSNMVVKS	CKGS
TGF-44	CGV	-	PQTLOPLPIIYYVG	-	RNV RVEQLSNMVVRA	CKGS
TGF-41	CGV	-	PQALEPLPIIYYVG	-	RKPKVEQLSNMIVRS	CKGS
TGF-45	CGV	-	PDVLEPLPIIYYVG	-	RTAKVEQLSNMVVRS	CKGS
GDF-9	SGV	-	PGKYSPLSVLTIEPD	-	GSIA YKEYEOMIATR	GTGR
Inhibin1	CGA	-	ALPGTMRPLHVRTTS	-	DGGYSFKYETVPNLLTQH	CGAI
Inhibin2A	CGV	-	PTKL R PMSMLY YODG	-	QNI I K KDI QNMIVEE	CGGS
Inhibin2B	CGI	-	PTKLSTMSMLYFODE	-	YNI V KROVPNMIVEE	CGGA
Inhibin2C	CGV	-	PTARRPLSLLYYDRO	-	SNIVKT D I POMVVEA	CGGS
MIS	CGV	-	PTATAGKLLISLSE	-	ERISAHHVPHMVA TE	CGGR
GDNF	CGR	-	PIAFODD - LSFLD	-	DNLVYHILRKHS AKR	CGGI
BMP-11	CGT	-	PTKMSPIMMLYFN DK	-	QQI I YGKI PGMVVD R	CGGS
GDF-8	SGV	-	PGKYSPLSVLTIEPD	-	GSIA YKEYEOMIATR	GTGR

09375337-084600

FIG. 5A

TGF-β Subgroup									
TGF-β1:	C	C	V	R	Q	L	Y	I	D
TGF-β2:	C	C	L	R	P	L	Y	I	D
TGF-β3:	C	C	C	V	R	P	L	Y	I
TGF-β4:	C	C	C	V	R	P	L	Y	I
TGF-β5:	C	C	C	V	K	P	L	Y	I
Pattern:	C	C	V	R	P	L	Y	I	D
Vg/dpp Subgroup									
dpp:	C	R	R	H	S	L	Y	V	D
Vg-1:	C	K	K	R	H	L	Y	V	E
Vg-1:	C	K	K	H	E	L	Y	V	S
60A:	C	Q	M	Q	T	L	Y	I	D
BMP-2A:	C	K	R	H	P	L	Y	V	D
DORSALIN:	C	R	R	T	S	L	H	V	N
BMP-2B/BMP-4:	C	R	R	H	S	L	Y	V	D
BMP-3:	C	A	R	R	Y	L	K	V	D
BMP-5:	C	K	K	H	E	L	Y	V	S
BMP-6:	C	R	K	H	E	L	Y	V	S
OP-1/BMP-7:	C	K	K	H	E	L	Y	V	S
OP-2:	C	R	K	H	E	L	Y	V	S
OP-3:	C	R	R	H	E	L	Y	V	S
Pattern:	C	n	n	r	r	L	Y	V	r
GDF Subgroup									
GDF-1:	C	R	T	R	R	L	H	V	S
GDF-3:	C	H	R	H	Q	L	F	I	N
GDF-9:	C	E	L	H	D	F	R	L	S
Pattern:	C	r	x	r	r	f	x	c	r
Inhibin Subgroup									
Inhibin α:	C	H	R	V	A	L	N	I	S
Inhibin βA:	C	C	K	K	Q	Q	F	F	V
Inhibin βB:	C	C	C	R	Q	Q	F	F	I
Pattern:	C	x	n	x	x	f	x	a	r
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+									
1	BETA				10	HELIX		FINGER 1	
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+									
20									
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+									
30									
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+									
RING KNOT_A									

FIG. 5B

TGF-β Subgroup									
TGF-β1:	P	Y	I	W	S	-	-	-	-
TGF-β2:	P	Y	L	W	S	-	-	-	-
TGF-β3:	P	Y	L	R	S	-	-	-	-
TGF-β4:	P	Y	I	W	S	-	-	-	-
TGF-β5:	P	Y	I	W	S	-	-	-	-
Pattern:	P	Y	C	W	S	-	-	-	-
Vg/dpp Subgroup									
dpp:	P	F	P	L	A	D	H	F	-
Vg-1:	P	Y	P	L	T	E	I	L	-
Vgr-1:	S	F	P	L	N	A	H	M	-
60A:	N	F	P	L	N	A	H	M	-
BMP-2A:	P	F	P	L	A	D	H	L	-
DORSALIN:	F	F	P	L	T	D	N	V	-
BMP-2B/BMP-4:	P	F	P	L	A	D	H	L	-
BMP-3:	Q	F	P	M	P	K	S	L	-
BMP-5:	S	F	P	L	N	A	H	M	-
BMP-6:	S	F	P	L	N	A	H	M	-
OP-1/BMP-7:	A	F	P	L	N	S	S	M	-
OP-2:	S	F	P	L	D	S	C	M	-
OP-3:	I	Y	P	L	N	S	C	M	-
Pattern:	X	F	P	L	X	X	X	b	-
GDF Subgroup									
GDF-1:	A	L	P	E	T	L	R	G	P
GDF-3:	P	F	S	M	T	T	Y	L	-
GDF-9:	P	R	A	V	R	H	R	Y	-
Pattern:	j	x	j	x	r	x	x	x	z
Inhibin Subgroup									
Inhibin α:	G	L	H	I	P	P	N	L	S
Inhibin βA:	P	S	H	I	A	G	T	S	G
Inhibin βB:	P	A	Y	L	A	G	V	P	G
Pattern:	j	x	e	c	j	j	x	x	j

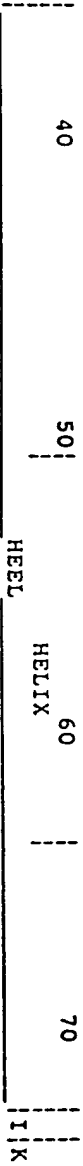


FIG. 5C

TGF-β Subgroup		80	90	100	110
TGF-β1:	V - - P Q A L E P L P I V Y Y V G - - R K P K V E E Q L S N M I V R S I C K C S				
TGF-β2:	V - - S Q D L E P L T I L Y Y I G - - K T P K I E Q L S N M I V K S I C K C S				
TGF-β3:	V - - P Q D L E P L T I L Y Y V G - - R T P K V E E Q L S N M I V K S I C K C S				
TGF-β4:	V - - P Q T L D P L P I I Y Y V G - - R T V K V E E Q L S N M I V R A I C K C S				
TGF-β5:	V - - P D V L E P L P I I Y Y V G - - R T V K V E E Q L S N M I V R S I C K C S				
Pattern:	V - - P Q X L E P L j I C Y Y V G - - R r j K V E E Q L S N M a V n S I C K C S				
Vg/dpp Subgroup		80	90	100	110
dpp:	V - - P T Q L D S V A M L Y L N D Q - - S T V V L K N Y Q E M T V V D E I C G C R				
Vg-1:	V - - P T K H S P I S M L F Y D D N N - - D N V V L K R H Y E N M A V D E I C G C R				
Vg-1:	V - - P T K L N A I S V L Y F D D N N - - S N V V I L K K X Y R N M A V V R A I C G C H				
60A:	V - - P T R L G A L P V L Y H L N D - - E N V V I L K K X Y R N M I V K S I C G C H				
BMP-2A:	V - - P T E L S A I S M L Y L D E N - - E K V V L K N Y Q D M H V V E G I C G C R				
DORSALIN:	V - - P T K L D A I S I L Y L D E N - - D K V V L K N Y Q L M H V V E G I C G C R				
BMP-2B/BMP-4:	V - - P T E L S A I S M L Y L D E N - - K N V V L K K V Y P N M T V V E S I C G C R				
BMP-3:	V - - P E K H S S L S I L F F D D E N - - S N V V I L K K X Y R N M A V V R A I C G C H				
BMP-5:	V - - P T K L N A I S V L Y F D D D N - - S N V V I L K K X Y R N M A V V R A I C G C H				
BMP-6:	V - - P T K L N A I S V L Y F D D D N - - S N V V I L K K X Y R N M A V V R A I C G C H				
OP-1/BMP-7:	V - - P T Q L N A I S V L Y F D D S - - S N V V I L K K X Y R N M A V V R A I C G C H				
OP-2:	V - - P T K L S A I S V L Y Y D S S - - N N V V I L R K K H R N M A V V Q A I C G C H				
OP-3:	V - - P T E L S A I S V L Y Y D S S - - N N V V I L R K K H R N M A V V Q A I C G C H				
Pattern:	V - - P T P L R A A S C L Y I F D M r z r r V a L n r Y p I M X V p j C G C r				
GDF Subgroup		80	90	100	110
GDF-1:	V - - P E R L S P I S V L F F D N S - - D N V V L R H Y E D M A V V D E I C G C R				
GDF-3:	V - - P T K L S P I S M L Y Q D S D - - K S I A Y L R H Y E D M A V V D E I C G C R				
GDF-9:	V - - P G K Y S P L S M L T I E P D - - G S I A Y L R H Y E D M A V V D E I C G C R				
Pattern:	V - - P X n f s p c s c l i x k x r - - X r a x f n r Y E D M a X r p C j C i x				
Inhibin Subgroup		80	90	100	110
Inhibin α:	A A L P G T M R P L H V R T T S D G G Y S F I K X E T V P N L L T Q H C A C I				
Inhibin βA:	V - - P T K L R P P M S M L Y Y D D G - - Y N I I V K K D V P N M I V E E I C G C A				
Inhibin βB:	I - - P T K L S T M S M L Y F D D E - - Y N I I V K K D V P N M I V E E I C G C A				
Pattern:	X z z P j r b r j b r c X i x r D X z x r f i x x p r a x n b c X o r C h C i x				
		BETA	LOOP	BETA	RING
			FINGER_2		KNOT2
					C

FIG. 6

TCF- β Subgroup
 Pattern: C C V R P L Y I D F R N D L G W K - W I H E P K G Y X A N P C X G j C
 Vg/dpp Subgroup
 Pattern: C n n r r L Y V r F r - D C G W r D M I A P P G Y X A d Y C r G k C
 GDF Subgroup
 Pattern: C r X r r f X c r F r - r c X W r r W a a A P r X d X j r d C r G r C
 Inhibin Subgroup
 Pattern: C X n X X f X a r F p - X c G W m r W I a X P j j d X X r Y C r G X C

1	BETA	10	HELIX	20	LOOP	30	BETA	RING
K			FINGER 1					KNOT_A

TCF- β Subgroup
 Pattern: P Y C W S - - - - X D T Q e S n V L j L Y N r X N P - - X A S A j P C C
 Vg/dpp Subgroup
 Pattern: X F P L X X X b - - - N j T N H A I a Q T L V r X C r z z - r X a P K j C C
 GDF Subgroup
 Pattern: j X j X r X X X z z z X j X X e j f c p X c c e X X X z z - P X X j r j X C
 Inhibin Subgroup
 Pattern: j X e c j j X X j X - - j X j X X j j X X X r X X X X z z z X j X X r j C C

40	50	60	70
	HEEL	HELIX	
			I K

TCF- β Subgroup
 Pattern: V - - P Q X L E P L j I c Y V V G - - R r j K V E Q L S N M a V n S C K C S
 Vg/dpp Subgroup
 Pattern: X - - P T P L r A a S C L Y f D m r z r r V a L n r Y p l M X V p j C G C r
 GDF Subgroup
 Pattern: V - - P X n f S P C S C L X X k X r - X r a X f n r Y E D M a X r p C j C X
 Inhibin Subgroup
 Pattern: X z z P j r b r j b r c X X X r D X z X r f X X p r a X N b c X o r C h C X

80	90	100	110
BETA	LOOP	BETA	RING
FINGER 2			KNOT2

Fig. 7A

pH2487

(N-terminal leader) collagen binding site OP-1 7-cysteine domain
 M T M I T N S L A S [W R E P S F M A L S] S S D Q R Q A C K K H E L Y V S F R D L
 ATGACCATGATTACGAATTCCTGGCCAGCTGGAGAGAGCCAGCTTCATGGCCTTAAGCAGCAGGACCCAGAGGCAGGCCTGTAAGAAGCAGAGCTGTATGTCAGCTTCGAGAGCTG
 EcoRI MscI PvuII HindIII AflII StuI

G W Q D W I I A P E G Y A A Y Y C E G E C A F P L N S Y M N A T N H A I V Q T L
 GGCTGGCAGGACTGGATCATCGCGCTGAAGCTACGGCGCTACTACTGTGAGGGGGAGTGTGCTTCCTCTGAACCTCTACATGAACGCCAGCCAGCCAGCCATCGTGCCAGAGCTG
 A1471

V H F I N P E T V P K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D
 GTCCACTTCATCAACCCGAAACGGTGCCCAAGCCCTGCTGTGCGCCCAAGCAGCTCAGCGCTATCTCGTCTCTACTTCGATGACAGCTCCAAAGTCATCTGGAAGAAATACGAAGAC
 AvaII BspI Eco47III DrdI

M V V E A C G C R
 ATGGTGGTGGAGCTTGTGGCTGCAGATAGCTCCTCCGAGAATTC
 HindIII PstI EcoRI

PH2440 His-6 attached at 35 residues upstream of first cysteine; poor activity!?

```

      10      20      30
-CCATGGCTGACAACCATCACCATCATCAACATATG
... K A D N H H H H H H H
NcoI:1                      NdeI:2

      40      50      60      70      80      90      100      110      120      130      140
GGGAGCAAACAGCGCAGCCAGAACCGCTCCAAGACGCCCAAGAACCGGAAGCCCTGCCGATGGCCAACGTGGCAGAGAACAGCAGCAGCGACCGAGGGCAGGCC
G S K Q R S Q N R S K T P K N Q E A L R M A H V A E N S S S D Q R Q A
                        BsaHI:2                      BglI:7      OP-1-exon5-----      StuI
MscIdcm:b

```

Fig. 7B

PR2521 FB leader, and 15 residues upstream from first cysteine

```

      10      20      30      40      50      60      70      80      90     100     110
ATGATCGAATTCATGGCTGACAACAAATTCACAAAGGAACAGGCAGAACGGCTTCTACGACATCTTGCACCTGCGGAACTGAAGCAACAGCAGCGTAACGGGCTTCATCCAAAGCGTG
MHTTELEFAHQAEDNNAKCEQDNIAEYLYLILHLPLNLELQRNNGFIQSL
EcoRI:1      MluI:1      BglII:1
              XbaI:b

      130      140      150      160      170      180      190
GAAGAGCGCGTCTCAGTCTGCGAATCTGCTAGCGGATGCGAAGTGAAGATGCGCAGGCAACGGAATCGGCG
LELPSSQSAHLALADAKKLLHDAQAPKSA
MheI:1      FspI:b

      300      310      320      330      340
ATGGGCAAGCTGGCAGAGAACAGCAGCAGCGAGCAGAGGCGAGGCGT
K A N V A E E E E D Q X Q A
BglII:7      OP-1-exon5-----      StuI
NcoI:1
XcmI:8
MscIdcm:b

```

Fig 7C

09375333-004689

pH2525 FB- and His6-⁺ r, retaining 35 residues upstream on first cysteine; good refolding

ATGATCGAATTCATGGCTGACAACTAATGACAGGAGCAGCAGAGGCGTTCTACGAGATCTTGCACCTGCGCAAGCTGAACGAGAGCAGCGTAAAGCGCTTCATCCAAAGCGTGAA
EcoRI:1 MluI:1 BglII:1
XbaI:b
GAAGAGCGGCTCTCAGTCTCGGAATCTGCTAGCGGATGCCAAGAACTGAACGATGGCAGGCAAGGAAATCGGGCATGGCTGACAAACCATCACCATCATCAACCATATG
MheI:1 FspI:b NcoI:1 NdeI:2
GGGAGCAACAGCGGCGGAGAGAGCGCTCCAGAGCGGCCAGAAACAGGAGCGGCTGGGATGGCCAAAGTGGCAGAGAACAGCAGCAGCGAGCGAGCGAGCGAGCGGCT
G S K Q R S Q R R S K T P K N Q E I L R M A N V A X X S S S D Q R Q A
HscI StuI

Fig 7D

05375333-051609

Fig. 7 E

pH2527 FB-His-6-truncated OP-1 with acid cleavage site

ATGATCGAATTTCATGGCTGACAACAAATTCACAGGAACAGCAGAACGGGTTCTACGAGATCTTGCACCTGCGAACTGAACGAAAGAGCAGGTTAACGGGCTTCATCCAAAGCGCTGAAA
 M I E F A H A D N S K I E N K E Q Q Q R D A F I E G I L F R E L F P R K L A G E E L Q R N G F I I Q S A L K
 EcoRI:1 HluI:1 BglII:1
 XmaI:b

GAAGAGCGGCTCTCACTCTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGGCGAGGCAACGAAATCGGATCATCATCAACATCACTACCTGGGATCC
 E E F S Q S A N L L A D A K K I L N D I A Q K A P K S D E R F E R A K S D F
 NheI:1 fspI:b BsaBI

ATGGCCAACTGGCAGAGAACAGCAGCAGGACGAGGCGAGGCT
 K A K V I K K S S S D Q R Q I
 MacI StuI
 BglI

Bam NcoI
 (XmaI
 acid cleav. site

FIG. 7(F)

H2528
FB-His6-CDMP-3

10 20 30 40 50 60 70 80 90 100 110 1
-CCATGATCGAATTCATGGCTGACACAAATTCACAGGAACAGCAGAACGGTTCTACAGATCTTGCACCTGCGGAACCTGAACGAAGAGCAGGGTAACGGCTTCATCCAAAGCC
...K I E F H A D N K F N K E Q Q H A F Y E I L N L P N L N E E Q R N G F I Q S

130 140 150 160 170 180 190 200 210 220 230 2
AAAGAAGAGCGGCTCTCAGTCTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATCGCGCAGGCACCGAAATCGGATCATCATCAACATCAACACTCGGATCCCATGGCGTTGGCGG
K E E P S Q S A N L L A D A K K L N D A Q A P K S D H E E E E E E S D P H A L A

250 260 270 280 290 300 310 320 330 340 350 36
ACCGGTACAGCGGCAGGCAGCGGGGAGGTGCGGGCAGAGGTCTAGGTGGAAGTGGTAGATCTCGCTGCGAGCGCCAGCGCGTTGCACTGGGACTTCAGGAGCTCGGCTGGGAGCGACTG
T R T A Q G S G G G A G R G H G R R G R S R C S R K P L E V D F K E L G W D D N

370 380 390 400 410 420 430 440 450 460 470 48
ATCATCGCGCGCGCTGGACTACGAGGGGTACCACTGCGAGGGGCTTTGGGACTTCCCTTTGGTTGGCACTGAGCGCCAGCAACCATGOCATCAITCAGAGCGCTGCTCAACTCCATGGC
I I A P L D Y E A Y H C E G L C D F P L R S H L E P T H E A I I Q T L L N S H A

490 500 510 520 530 540 550 560 570 580 590 60
CCAGACGGGGGGCGCTCCTGCTGTGTGCGAGGGGGCTCAGCGCCATCAGCATCTCTACATGAGCGGGGGCAACAGTTGTCTACAGCAATACGAGGACATGGTGGTGGAGGG
P D A A P A S C C V P A R L S P I S I L Y I D A A N N V V Y K Q Y E D H V V E A

610 620 630 640 650
TGCGGCTGTAGGTAAGCTTGTGGCTGCAGATAGCTCCTCCGAGAAATTC
C G C R *

Fig. 7(G)

pH2469 truncated, good ROS activity; 14 original residues upstream of first cysteine

```
      10      20      30      40
-CCATGGCCAACGTGGCAGAGAACAGCAGCAGCGACCAGAGGCAGGCC
... M A N V A E N S S S D Q R Q A
  NcoI BglI:7      OP-1-exon5-----      StuI
MscIdcm:b
```


Fig. 7(H)

ph2510 Collagen site inserted 7 residues upstream of cysteine; good expression, refold

```

      10      20      30      40      50      60      70      80      90      100      110      120
ATGTCACGGGGAGCAACAGCGCAGCCAGAACCCGCTCCAGACGGCCCAAGAACAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCAGCGAOCAGAGGCCAG
M S T C S K Q R S Q K R S K T P K N Q K A L R M A S Y R P S F M A L S S S D Q R Q
      BsaHI:2                      BpmI+      HindIII:1      AclIII:1      BfrI:1      StuI
                                MscIdcm:b      PvuII

```

Fig. 7(I)

ph2523 collagen peptide, and spacer added at 13 residues upstream from 1st cysteine

```

      10      20      30      40      50      60      70      80      90      100      110      120
ATGTCACGGGGAGCAACAGCGCAGCCAGAACCCGCTCCAGACGGCCCAAGAACAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCAGCGAOCAGAGGCCAG
M S T C S K Q R S Q K R S K T P K N Q K A L R M A S Y R P S F M A L S S S D Q R Q
      BsaHI:2                      BpmI+      HindIII:1      AclIII:1      BfrI:1      StuI
                                MscIdcm:b      PvuII:b

```

```

      140      150      160
AACGTGGCAGAGAACAGCGCAGCGAOCAGAGGCCAGGCC
M V A E N S S S D Q R Q A
      OP-1-exon5-----      StuI
.....

```

Duplication

Fig. 7(J)

ph2524 Hexa-His, collagen peptide, spacer added at 13 residues upstream from 1st cysteine

```

      10      20      30
--CCATGGCTGACAAOCATCAOCATCATCAOCATATG
... M A D N K K K K K K M
NcoI:1                      NdeI:2

```

```

      40      50      60      70      80      90      100      110      120      130      140      150
GGGAGCCAAACAGCGCGCAGCAGAACCTCCAGACGGCCCAAGAACAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCAGCGAOCAGAGGCCAG
C S K Q R S Q N S K T P K N Q K A L R M A S Y R P S F M A L S S S D Q R Q
      BsaHI:2                      BpmI+      HindIII:1      AclIII:1      BfrI:1      StuI
                                MscIdcm:b      PvuII:b

```

```

      160      160      180      170
AACGTGGCAGAGAACAGCGCAGCGAOCAGAGGCCAGGCC
M V A E N S S S D Q R Q A
      OP-1-exon5-----      StuI
.....

```

Duplication

Fig. 8

7-cysteine domain of OP-1

finger-1

TGTAAAGACGAGCTGTATGTACAGCTTCGAGACCTGGGCTGGCAGACTGGATCATCGCGCTGAAGGCTACGCCCTACTACTGTAGGGG
C K K H E L Y V S F R D L G W Q D W I I A P E G Y A A Y Y C E G

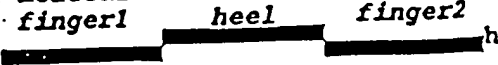

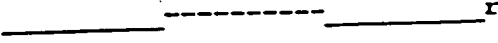



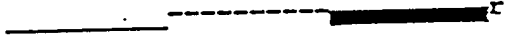





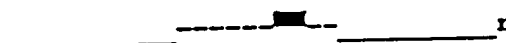


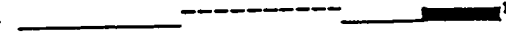

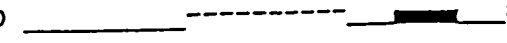
heel

GAGTGTGCCTTCCCTCTGAACCTCCTACATGAACGCCACCAACGCCCATCGTGCCAGACGCTGGTCCACTTCATCAACCCGGAACGGTGCCCAAGCCCTGC
E C A F P L N S Y M N A T N H A I V Q T L V H F I N P E T V P K P C

finger-2

TGTGCGCCACGAGCTCAATGCCATCTCCGTCCTTACTTCGATGACAGCTCCAACGTCATCCTGAAGAAATACAGAAACATGGTGGTCCGGGCTGTGGCTGCCAC
C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H

Figure 9A
OP-1 chimerics with CDMP-2 or with BMP-2

		refolding	activity (cell based)
Parental molecules:			
OP-1		(-)	+++ (*)
BMP-2		+++	+++
CDMP-2		++++	+/-
replacing finger-1 or heel:			
H2383		+/-	N/A
H2362		+	N/A
H2360		+	N/A
H2331		+	N/A
replacing finger-2 or heel:			
H2389		+++	+++
H2471		+++	+++
H2388		+++	+/-
H2410		+++	+++
H2429		+/-	N/A
changing patches of residues:			
H2381		+++	N/A
H2390		+	N/A
H2396		+	N/A
H2421		+/-	N/A
paired changes in finger-2:			
H2418		+++	++
H2420		++++	+/-

0075333-004655

[illegible]

OP-1 mutants with C-terminal arginine instead of histidine:

H2247	[REDACTED]	[REDACTED]	[REDACTED]	r	+	+++
			25,26,30			
H2233	[REDACTED]	[REDACTED]	[REDACTED]	ed, e, r	+	+++

Balancing of charged residues in finger-2 of OP-1 mutants:

Strain	Genotype	Phenotype	Notes
H2406	vktp ^{1,4,6,7} r	+/-	N/A
H2443	vktp ^{1,4,6,7} ed ^{25,26} r	+++	
H2447	ves ^{1,4,6} ede ^{25,26,30} r	+++	
H2433	k ⁴ r	+/-	N/A
H2456	es ^{4,6} ede ^{25,26,30} r	+++	

Correlation of Refolding Efficiency and Charged Amino Acids
in the TGF- β (Seven Cysteine) Domain

protein	finger-1	CXGXC	heel	finger-2	CXCX C-term	Total of charged residues (+), (-) = total	negative charges, finger-2	net charges, finger-2	refolding efficiency
OP-1	3+, 4-	2-	1+, 1-	4+, 2-	0	8+, 9- = 17	2-	2+	+/-
H2247	3+, 4-	2-	1+, 1-	4+, 2-	1+	9+, 9- = 18	2-	2+	+
H2447	3+, 4-	2-	1+, 1-	2+, 6-	1+	7+, 12- = 19	6-	4-	+++
BMP-3	4+, 4-	0	3+, 1-	3+, 4-	1+	11+, 9- = 20	4-	1-	+++
BMP-2	2+, 3-	1-	2+, 1-	2+, 6-	1+	7+, 11- = 18	6-	4-	+++
GDF-5	3+, 5-	1-	1+, 4-	2+, 4-	1+	6+, 14- = 20	4-	2-	+++
CDMP-2	3+, 5-	1-	1+, 3-	2+, 4-	1+	6+, 13- = 19	4-	2-	+++
GDNF	2+, 4-	0	6+, 4-	5+, 5-	0	13+, 13- = 26	5-	0	+++
TGF- β 1	5+, 3-	0	1+, 1-	5+, 2-	1+	11+, 6- = 17	2-	3+	+/-
TGF- β 2	5+, 3-	0	1+, 2-	4+, 3-	1+	10+, 8- = 18	3-	1+	+/-

Figure 10

Fig. 11

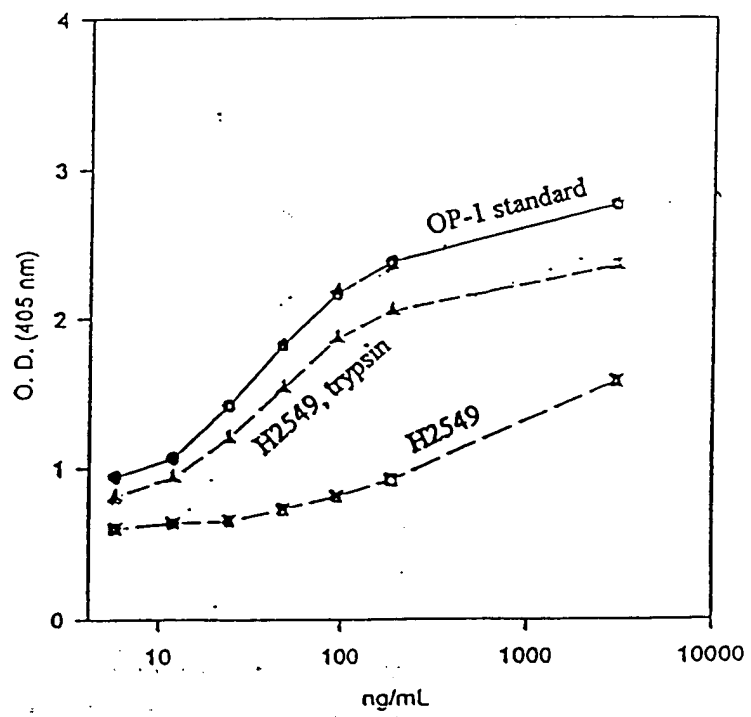


Fig. 12

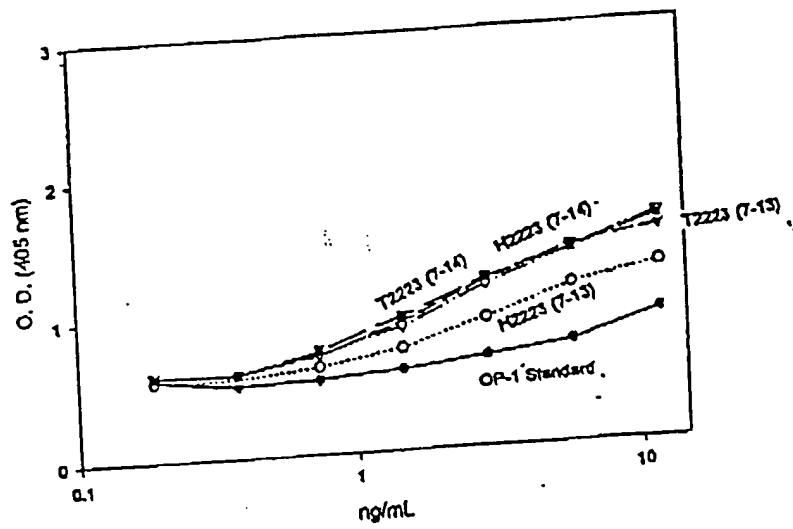


Fig. 13A

13B

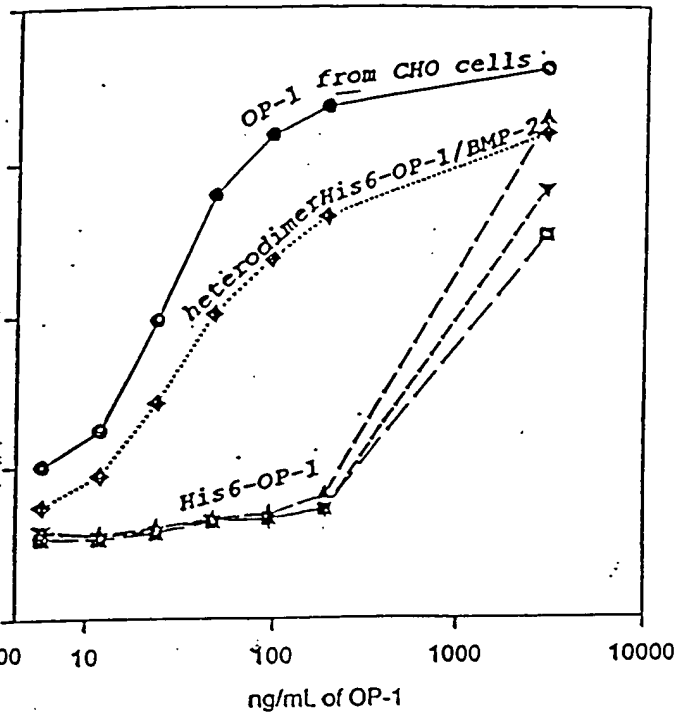
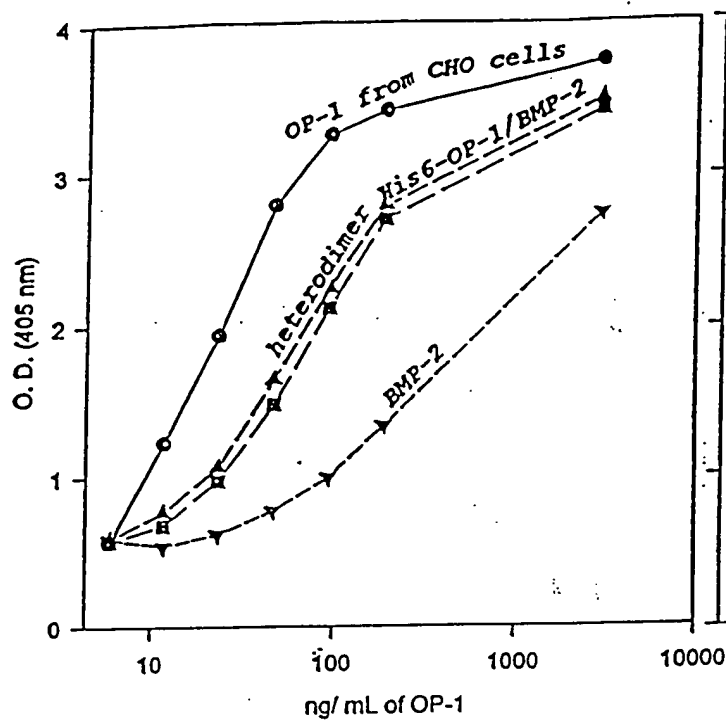
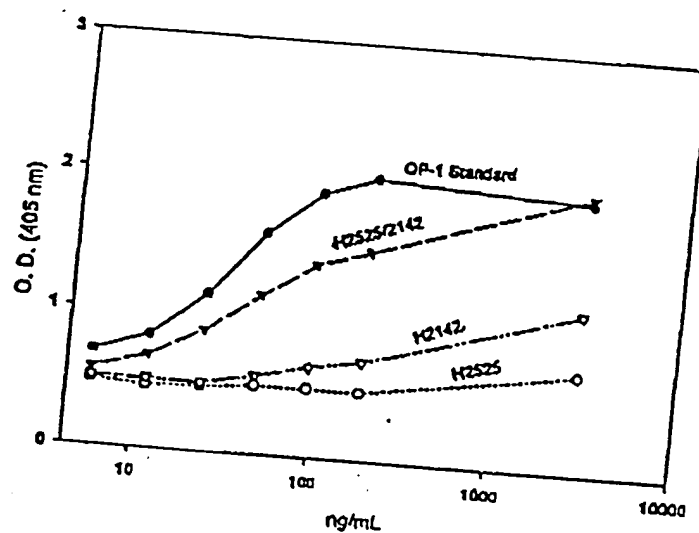


Fig. 14



		Changes	
		Folding	ROS
OP-1	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H	-	5 (-)
2421	P T C C V P T R L S P I S I L F I D A S N N V V L K K Y R N M V V R A C G C R	(+)	(+)
2406	N S C C V P T K L T P I S I L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	(+)
2410	N S C C V P T E L S A I S M L Y L D E N E K V V L K N Y Q D M V V E G C G C R	3+	4 (18)
2247	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	3 (1)
2234	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V R A C G C R	(+)	3 (3)
2233	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	1+	3 (4)
2418	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V E S C G C R	3+	2 (10)
2443	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V R S C G C R	3+	(9)
2447	N S C C V P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	2 (9)
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3 (8)
2456	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	3 (6)
2460	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	(5)
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3 (8)
2449	K P C C A P T E L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	2+	3 (2)
2467	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	(3)
2464	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	(2)

FIGURE 15

Finger-2 sequences of OP-1 mutants and their folding efficiencies and biological activities in the ROS cell based alkaline phosphatase assay.